(19) BUNDESREPUBLIK DEUTSCHLAND

DEUTSCHES PATENT- UND MARKENAMT

(12) Offenlegungsschrift

(10) DE 199 52 723 A 1

(51) Int. Cl.[7]:
C 12 M 1/34
C 12 M 1/38
C 12 Q 1/68

(21) Aktenzeichen: 199 52 723.7
(22) Anmeldetag: 26. 10. 1999
(43) Offenlegungstag: 10. 5. 2001

D5

DE 199 52 723 A 1

(71) Anmelder:
Epigenomics AG, 10435 Berlin, DE

(74) Vertreter:
Schubert, K., Dipl.-Chem. Dr.rer.nat., Pat.-Anw., 10119 Berlin

(72) Erfinder:
Berlin, Kurt, Dr., 14532 Stahnsdorf, DE

(56) Entgegenhaltungen:

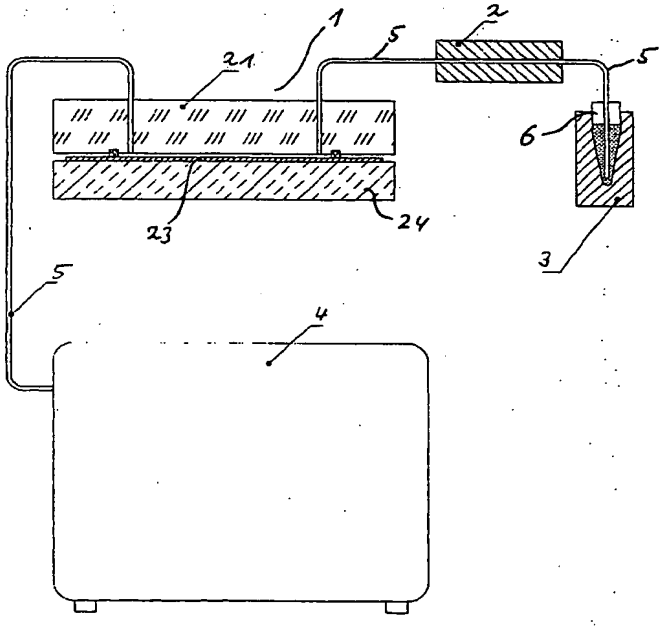
US	59 22 591
US	58 40 573
US	54 66 603
US	53 60 741
US	51 00 775
WO	97 12 063 A1

Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen

Prüfungsantrag gem. § 44 PatG ist gestellt

(54) Vorrichtung und Verfahren zur Hybridisierung doppelsträngiger DNA-Proben an Oligomer-Arrays

(57) Beschrieben wird eine Vorrichtung zur Hybridisierung doppelsträniger DNA-Proben an Oligomer-Arrays, umfassend mindestens eine in zwei Richtungen fördernde Pumpe (4, 14), eine geschlossene Hybridisierungskammer (1, 11), ein Kühlelement (2, 12) und ein Heizelement (3, 13), wobei die einzelnen Komponenten in der oben genannten Reihenfolge jeweils miteinander durch Flüssigkeiten fördernde Leitungswege (5, 15) verbunden sind.



DE 199 52 723 A 1

EXPRESS MAIL LABEL NO.: EV 481671870 US

Beschreibung

Die Erfindung betrifft eine Vorrichtung und ein Verfahren zur Hybridisierung doppelsträngiger DNA-Proben an Oligomer-Arrays.

Hybridisierungen von Proben-DNA auf Oligomer Chips, beispielsweise Oligonukleotid-Arrays, werden zum Nachweis bestimmter Sequenzen in der Proben-DNA durchgeführt. Ein möglicher Ansatz, "Sequencing by hybidisation(SBH)", ermittelt dabei sogar die vollständige Sequenz der Proben-DNA oder zumindest großer Teile davon. Alle spezifische Hybridisierungen werden jedoch auch ausgeführt, um bestimmte Veränderungen in der Proben-DNA, z. B. Punktmutationen, nachzuweisen. Die Proben-DNA liegt jedoch, da sie zumeist vorher mittels PCR amplifiziert wurde, in der Regel doppelsträngig vor, damit steht sie im wesentlichen einer Hybridisierung mit den Oligomeren nicht mehr zur Verfügung. Die vorliegende Erfindung beschreibt eine Vorrichtung, die zur effizienten Hybridisierung doppelsträngiger DNA an Oligomer Arrays dient.

Im Stand der Technik sind eine Reihe von Hybridisierungskammern bekannt. So beschreiben die US-A 5,100,775, US-A 5,360,741 oder US-A 5,466, 603 Hybridisierungskammern, welche für die unterschiedlichsten Zwecke und Bedürfnisse angepaßt sind. Derartige Hybridisierungskammern werden inzwischen vielfach kommerziell angeboten, sind aber im Allgemeinen nicht separat temperierbar. Auch sind für die Aufnahme von Objektträgern geeignete Hybridisierungskammern bekannt. Weiterhin gibt es an den Rändern selbstklebende Folien, die durch das Aufkleben auf Objektträger Hybridisierungskammern bilden können. Bekannt ist auch eine pneumatisch ansteuerbare und temperierbare Hybridisierungskammer, bei der die Hybridisierungseigenschaften durch die Bewegung der Hybridisierungsflüssigkeit verbessert sein sollen.

Alle diese Vorrichtungen erfordern jedoch, daß die doppelsträngige Proben-DNA vor der Hybridisierung entweder thermisch denaturiert wird, einer der Stränge selektiv zuvor abgetrennt wird (z. B. kann ein Primer in der PCR mit Biotin markiert sein und in einem nachfolgenden Schritt durch Bindung an Streptavidin selektiv ein Strang aus der Lösung entfernt werden) oder aber durch enzymatische Verfahren ein Strang im Überschuß erzeugt wird, damit die an den Oligomer-Array zu hybridisierenden Abschnitte nicht von komplementären Strängen blockiert werden. Diese Verfahren stellen nicht nur einen zusätzlichen Schritt dar, sondern sie sind auch speziell im Falle des Biotins teuer und im Falle enzymatischer Reaktionen oftmals schlecht reproduzierbar. Sollen beide Stränge jedoch durch den Oligomer-Array nachgewiesen werden, so kommt ohnehin nur die thermische Denaturierung in Frage. Das Problem dabei ist jedoch das sogenannte Reannealing, das heißt daß die komplementären Stränge nach dem Denaturieren wiederum miteinander hybridisieren, was schneller als eine Hybridisierung mit Oligomeren auf dem Chip erfolgen kann. Dieses Problem wird auch durch einmalige Denaturierung nicht gelöst. Wird dagegen in der Kammer denaturiert, so werden bereits an den Oligomer Array hybridisierte DNA-Fragmente ebenfalls wieder abgelöst.

Aufgabe der vorliegenden Erfindung ist es daher, eine Vorrichtung zu schaffen, welche die Nachteile des Standes der Technik überwindet und eine effektive Hybridisierung von doppelsträngigen DNA-Proben ermöglicht.

Die Aufgabe wird durch die Merkmale des Anspruchs 1 gelöst. Vorteilhafte Ausgestaltungen sind in den abhängigen Unteransprüchen gekennzeichnet.

Die Aufgabe wird also durch eine Vorrichtung zur Hybridisierung doppelsträngiger DNA-Proben an Oligomer-Arrays (Oligomer-Chips) gelöst, umfassend mindestens eine in zwei Richtungen fördernde Pumpe, eine geschlossene Hybridisierungskammer, ein Kühlelement und ein Heizelement, wobei die einzelnen Komponenten in der oben genannten Reihenfolge jeweils miteinander durch Flüssigkeiten fördernde Leitungswege verbunden sind.

Die hier beschriebene Vorrichtung ermöglicht ein periodisches Denaturieren der DNA-Probe, ohne eine Ablösung bereits an Oligomere hybridisierter DNA zu erzeugen und überwindet damit die im Stand der Technik erwähnten Probleme. Dadurch, daß die DNA-Probe vor dem Aufbringen auf den Chip thermisch denaturiert und dann plötzlich abgekühlt wird, liegt sie bei Kontaktierung mit dem Chip überwiegend in einzelsträngiger Form vor. Damit steht ein großer Teil der ansonsten doppelsträngigen DNA-Probe für Hybridisierungen mit dem Oligomer-Array zur Verfügung. Durch Hin- und Herpumpen der Probenflüssigkeit stellt die Vorrichtung sicher, daß dieser Vorgang so häufig wiederholt wird, bis ein ausreichender Teil der doppelsträngigen DNA-Probe an die Oligomere des Chips hybridisiert hat. Zugleich findet durch diesen Prozeß ein Mischen in der Kammer während der Hybridisierungsphase statt.

Erfindungsgemäß ist bevorzugt, daß die Pumpe eine peristaltische Pumpe, eine Schlauchpumpe oder eine Kolbenpumpe ist. Die Pumpe soll in der Lage sein, kleine Flüssigkeitsmengen präzise in Saug- und Druckrichtung zu fördern. Dies kann erfindungsgemäß auch durch eine dem Fachmann bekannte Schaltung mittels Ventilen, wie Mehrwegventilen erfolgen, welche ihrerseits wieder extern ansteuer- und/oder regelbar sind.

Besonders bevorzugt ist es dabei, daß die Pumpe programmierbar oder durch ein Computer gesteuert ist. Derartige Steuerungen und/oder Computerprogramme sind dem Fachmann an sich bekannt.

Erfindungsgemäß bevorzugt ist es ferner, daß die Hybridisierungskammer mindestens einen Deckel, mit durch diesen hindurch geführten Ein-/Auslaßkanälen, und einen Temperierblock, mit einem darauf auflegbaren oder festlegbaren Oligomer-Array, umfaßt.

Dabei ist insbesondere bevorzugt, daß weiterhin ein Kühlkörper vorhanden ist, auf welchem der Temperierblock angeordnet ist.

Erfindungsgemäß bevorzugt ist ferner eine Vorrichtung, wobei das Volumen der Hybridisierungskammer bei eingelegtem Oligomer-Chip weniger als 200 µl beträgt.

Besonders vorteilhaft ist es, daß die Hybridisierungskammer für die Aufnahme handelsüblicher Objektträger oder Mikroskopobjekträger vorgerichtet ist.

Erfindungsgemäß bevorzugt ist es auch, daß das Kühlelement den Leitungsweg fest umschließt.

Bevorzugt ist es ferner, daß das Heizelement den Leitungsweg fest umschließt und daß der Leitungsweg mit seinem offenen Ende aus dem Heizelement herausragt. Dabei ist es alternativ bevorzugt, daß das Heizelement ein Probengefäß mindestens teilweise umschließt und das der Leitungsweg mit seinem offenen Ende in die in dem Probengefäß vorhandenen Probenlösung eintaucht und daß dieser Leitungsweg gegebenenfalls bis auf die Innenseite des Bodens des Probengefäßes geführt ist.

Bevorzugt ist es ferner, daß die Leitungswege Schläuche sind und vorzugsweise aus einem inerten Material, Silikonkautschuken, Polytetrafluorethylen, Polyvinylchlorid, Polyethylen und/oder Edelstahl bestehen. Auch andere inerte Materialien kommen in Frage und sind dem Fachmann bekannt.

Ganz besonders bevorzugt ist es, daß die Hybridisierungskammer, das Kühlelement, das Heizelement und der Temperierblock unabhängig voneinander temperierbar sind.

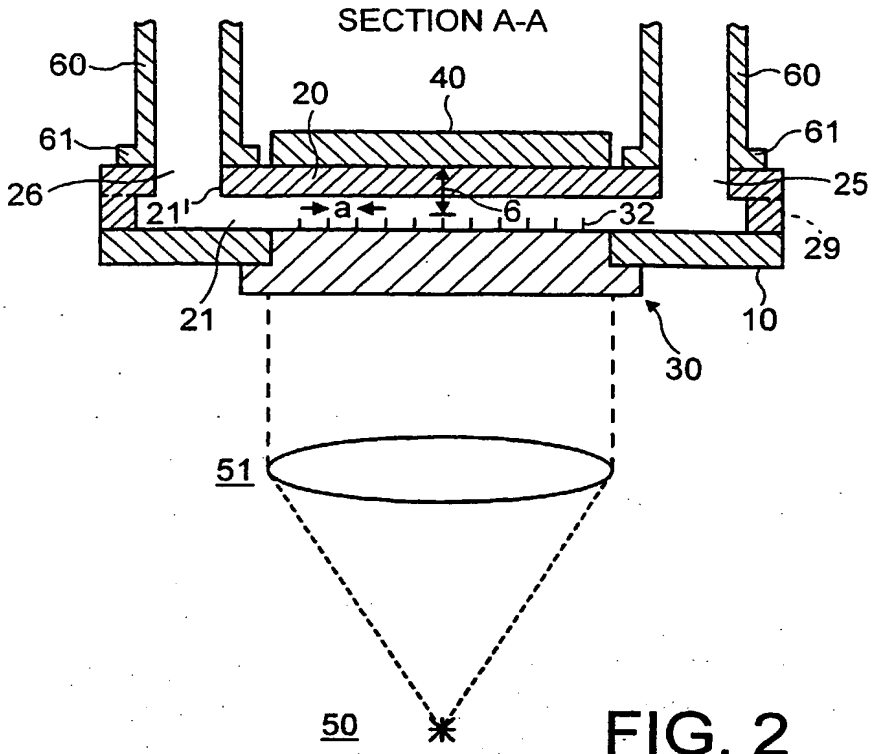


FIG. 2

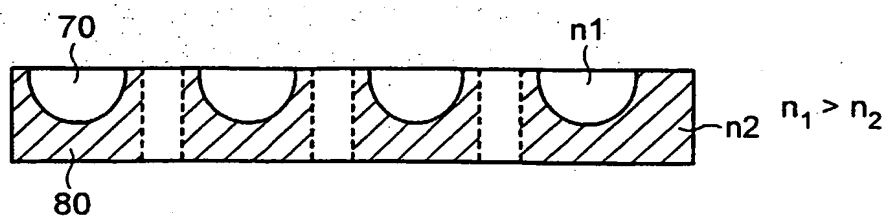


FIG. 3

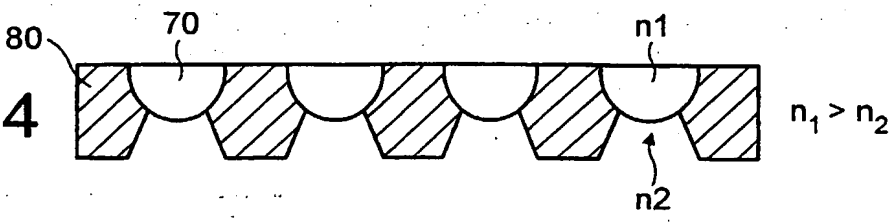


FIG. 4

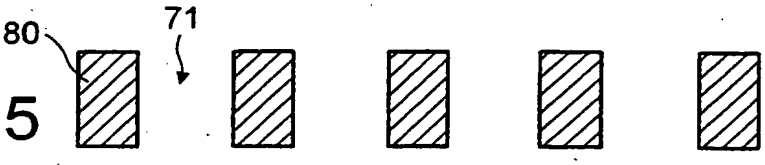


FIG. 5

2368903

ANALYSIS OF BIOLOGICAL AND BIOCHEMICAL ASSAYS

The present invention describes an apparatus and method for analysing a biological or biochemical assay. Samples to be analysed may form an array on a substrate or be located within a flow cell.

Technologies in which a large number of different biological or biochemical samples are attached to a substrate or chip in a two dimensional array have received significant interest in recent years. Applications for such arrays range from assays for determining binding reactions between proteins to studying hybridization reactions between nucleotide or gene sequences which can be used to study gene function.

The advantage of the array-based technologies is their potential scalability for analyzing a high number of probes within a short amount of time. As such they represent an extension of multi-well-plate based assays performed by scientists largely manually in the past. The interest in large-scale substrate of chip based array technology has been fueled by the development of techniques of producing such arrays efficiently using lithographic or other techniques. A method for making very large arrays of polymer sequences, including polypeptides and polynucleotides on very small substrates has been disclosed, for example, in US 5,143,854, the disclosure of which is incorporated herein by reference.

Analysing the arrays thus produced normally requires the performance of one or several biochemical reactions followed by optical imaging performed on the array. The imaging has typically been achieved either by means of illuminating the array with a light source and detecting an image of the array.

Various such detection techniques are described in US5,631,734, US5,834,758, WO9913318, and US6,114,122. All of these disclosures are incorporated herein by reference. Most of the prior art techniques are based on shining an excitation beam of light onto the array from the side of the chip containing the array. Reflected or fluorescently emitted light is then detected by scanning each sample in the array in

sequence. This is typically done by using a bulk optics arrangement comprising one or more focusing lenses and a photodetector or photomultipliers. The scanning of the array is typically performed by moving the array on a x-y stage, one vertex at a time. It has been noted in the past that such scanning detection may be very time consuming when large-scale arrays are scanned.

More recently, with the advent of high-resolution CCD cameras it has become possible to image the whole array or sections of the array at a time. When used with small size array features it has generally been necessary with the prior art to use confocal microscopy techniques in combination with the specific detection method used. Although high resolution with good depth discrimination can be achieved this way, the field of view is limited when using this technique. A typical 40x microscope objective that has a resolution of 0.25um has a field size of only about 500um. Thus confocal microscope techniques are inadequate for application requiring high resolution and a large field of view simultaneously.

The invention provides a method of analysing a biological or biochemical assay which method comprises outputting light simultaneously from a plurality of test locations in a test sample and detecting simultaneously optical signals from said test locations, said detecting comprising providing a plurality of optical detectors, one for each test location, and positioning said detectors in an array overlying the sample so that the position of each detector in the array has a one to one correspondence with its respective test location.

The invention also provides a method of analysing a biological or biochemical assay in a flow cell having a flow cavity enclosed by boundary walls one of which provides a flat output window transparent to optical signals to be output from the cell, which method is characterised by outputting light from a plurality of test locations in the assay to provide optical signals which are transmitted through the window of the flow cell and detecting said optical signals by incidence on respective light detectors in an array of light detectors forming an array of pixel locations respectively corresponding to said test locations and positioned close to said output window without requiring an optical imaging system to focus an image of the array of test locations onto the array of light detecting devices.

Said optical signals may be generated from samples on a sample chip located in an aperture in a wall of the flow cell opposite said output window and parallel thereto.

Said light signals are generated from samples within the cavity of the flow cell, the depth of samples within the flow cell perpendicular to said output window being controlled to avoid more than one sample being stacked on the same output path.

The invention also provides an apparatus for analysing a biological or biochemical assay which apparatus comprises a support structure for a test sample having an array of test locations, said support structure comprising at least one planar surface, and said apparatus further comprising an optical detector for detecting optical signals from said sample, the detector having a planar array of separate light detecting devices each corresponding to a pixel in the detector output and each having a position on the array corresponding to a respective desired test location, said planar array of light detecting devices facing said first planar surface substantially in parallel and in sufficient proximity thereto with said test locations being in between the said first planar surface and said array of light detecting devices, such that light from an array of test locations in the assay passes from the sample to a respective pixel location in the detector array without requiring an optical imaging system to focus an image of the array of test locations onto the array of light detecting devices.

Said support structure may comprise an output window transparent to said optical signals to be output from the sample and wherein said detecting devices are being located in a planar array in contact with said window.

Said support structure may comprise an output window transparent to said optical signals to be output from the sample and wherein said detecting devices are being located in a planar array facing said window in close proximity thereto.

Said detecting devices may be located in a planar array with a pitch separation in the array corresponding to the pitch separation of test locations in the sample and the array of detecting devices being located such that the average distance

between the planar array of detecting devices and the test locations is less than 5 times said pitch separation.

The average distance between the planar array of detecting devices and the test locations may preferably be less than 2 times said pitch separation.

Said support may structure include a flow cell and said window may be provided by a transparent wall region of the cell.

A sample chip holding an array of biological or biochemical samples may be releasably secured to the flow cell adjacent an aperture in a wall of the cell opposite said output window such that said samples are exposed in the cavity of the flow cell

The apparatus may comprise a stack of planar elements in contact with each other at least some of said planar elements forming the flow cell and a further planar element providing the array of optical detectors.

A sample chip may form one of said planar elements.

The invention further provides an apparatus for performing and reading out a biological or biochemical assay in a flow cell the flow cell being contained between an top surface, side walls and a bottom surface, the bottom surface comprising the top surface of a sample chip, the sample chip comprising biological or biochemical samples in an array characterized in that an array of optical detectors is directly apposed to the top surface of said flow cell to read out optical signals emitted from the samples on the sample chip such that substantially one vertex in the array of samples is imaged onto one vertex or pixel of the array of detectors.

Embodiments of the present invention shall now be described with reference to the accompanying drawings in which:

Figure 1 is an exploded perspective view of a flow cell system of the present invention,

Figure 2 is a cross section along surface A-A of the assembled flow cell,

Figure 3 is a cross section of a lens array, which may be used in preferred embodiments of the flow cell system of the invention,

Figure 4 is a cross section of an alternative of the lens array, which may be used in preferred embodiments of the flow cell system of the invention, and

Figure 5 is a cross section of an array of holes in a substrate, which may be used in preferred embodiments of the flow cell system of the invention

In Figure 1 a flow cell is being formed by a bottom substrate 10 with a recess 11 receiving an assay chip 30 and a micromachined lid 20. The assay chip 30 has alignment features 31 which mate with alignment features 12 formed around the recess 11 of the bottom substrate 10 and carries an array of biochemical or biological samples 32 on its top surface 33. The samples may for example be various oligonucleotides which are attached at predetermined locations in an array either at their 3' or at their 5' end. Alternatively the samples may be polypeptides or functional proteins that are attached at predetermined locations in the array. The lid 20 is recessed by micromachining the monolithic substrate 20, e.g. by means of a reactive ion etch to form a recess 21 with sidewalls 22, defining the height of the flow cell. This height is between 5 and 100 microns, or alternatively it may be between 20 and 50 microns. The lid 20, the bottom substrate 10 and the chip 30 may each be made from materials such as silica or quartz glass or, depending on the wavelength studied, silicon or silicon on insulator. The recess 21 further defines inlet and outlet regions at either end of a central assay and detection region of the flow cell. Inlet and outlet ports 25 and 26 are provided by vias that extend from the in and outlet regions of the lid directly through the lid to its top side. These vias can be formed by means of the same or similar micromachining techniques as the recess 21.

The bottom surface 21' of the recess 21 forms the top surface of the flow cell, which, in its center, has a detection region 27. The detection region 27 is

transparent at a wavelengths or band of wavelength to be detected by an array of detectors 41. The array of detectors 41 is provided on the bottom surface 42 of a detector chip 40 which may be a CCD chip. The detector chip 40 is mounted at a predetermined location directly above the detection region 27 on top of the lid 20 with the bottom surface facing downwards.

In an alternative embodiment the lid 20 may be made of two parts, i.e. a top substrate whose bottom surface defines the surface 21' of the recess and a bottom substrate, which is bonded to the top substrate, whereby the bottom substrate is machined through to provide the side walls for the recess 21 once it is bonded to the top substrate of the lid. Such an optional arrangement is indicated in Fig. 1 by the dashed line 29 which represents the bond line between the bottom substrate and the top substrate of the lid 20. Such an arrangement has the advantage that a high optical quality may be achieved for the surface 21', e.g. by polishing, which may be required for the detection window 27.

In Figure 2, the closed flow cell is shown where the lid 20 engages with the bottom substrate 10 by means of first fiduciary marks or alignment features 24 (shown in Figure 1) on the bottom surface 28 of the lid 20 and second fiduciary marks of alignment features 13 provided top surface 14 of the bottom substrate 10. At the same time the bottom substrate 10 engages the chip 30 carrying the array of biochemichal or biological samples 32 as described before. The pitch between samples in the array is a distance *a*, which is substantially the same in magnitude as the pitch of the vertixes or pixels in the detector chip 40 so that there is a one to one correspondence between the positions of detectors in the detector array and the respective test locations in the test sample. The distance between light emitting portions of the samples in the array 32 and the pixels in the array is shown as *b*, which is preferably similar in magnitude to the distance *a*. In addition, flanged tubes 60 are shown, which connect via flanges 61 to the inlet and outlet ports 25, 26. A fluid tight connection may be achieved by means of a press fit, a permanent connection, e.g. by means of glue, resin or solder or similar means well known in the art.

If a wafer fabrication method is used for making the lid 20 and the bottom substrate 10, direct wafer-to-wafer bonding may be used to bond the lid 20 to the bottom substrate 10. Other bonding techniques may be used, e.g. by means of a UV cured resin or glue. The engagement between the bottom substrate 10 and the assay chip 30 is non-permanent, but the alignment features are made such that a fluid tight press seal can be made between the bottom substrate and the chip when they engage with each other.

Due to the predetermined alignment of the detector chip 40 with the lid 20, the alignment of the lid 20 with the bottom substrate 10 and the alignment of the bottom substrate with the assay chip 30 the array of biological or biochemical samples 32 is aligned in parallel with the array of detectors 41 with each vertex in the sample array 32 being directly opposite one vertex or pixel in the array of detectors 41.

Depending on the application, the flow cell may have a temperature control means (not shown), e.g. comprising a thermistor and a Peltier device to enable control of the flow cell and its contents as desired during operation.

The chip 30 is made of a material that is transparent at a first wavelength emitted by a light source 50. The light source 50 emits light at the first wavelength or band of wavelengths. Depending on the wavelengths of light used in the system appropriate materials for forming the chip are for example, silica, quartz glass, silicon or silicon on insulator. The light is collimated by a lens 51 onto the chip 30 and is transmitted trough the chip on to the array of biochemical or biological samples 32 located on the top surface of the chip 33.

Previous to the light exposure a biological or biochemical reaction has been performed on the samples in the array in the flow cell such that samples in the array have been labeled with a fluorescent marker wherever a specific reaction has occurred. The fluorescent marker may be for example R-phycoerythrin (R-PE), allophycocyanin (APC), flourescinisothiocyanate (FITC), rhodamine, or texas red. The light transmitted through the chip which is at a first wavelength or band of wavelengths causes the fluorescent markers to fluoresce at a second wavelength or band of wavelengths where they are bound to the samples in the array. At each

vertex in the array fluorescent light emitted from markers is detected by the vertex or pixel in the CCD or other detector chip that is directly apposed to this vertex and the detection of fluorescence is possible at all vertexes in the array at the same time.

Since the array of detectors 41 is directly apposed to the top surface of the flow cell 21 and its vertexes or pixels are matched with those in the sample array substantially one to one, this provides for a very compact arrangement for a flow cell with high resolution and an view field that is not limited by the resolution required. In the specific example where an external light source 50 is used to cause fluorescence of the samples by putting the light source on the opposite side of the chip 30 from the detector 40 and illuminating the sample array 32 through the chip 30 allows easier management of components in a workstation and helps to reduce the size of the entire device.

In order to reduce crosstalk between signals received from adjacent vertexes on the sample array it is preferable that the height of the flow cell or the distance between the marker molecules in the sample array and the pixels in the array of detectors is similar in magnitude to the spacing between vertices in the array.

In order to improve the detection of a fluorescent signal at a second wavelength in response to a primary excitation of light a first wavelength it is possible to provide the detector 40 with a bandpass filter by means of an optical coating deposited on the bottom surface 42 of the detector chip 40 or on the detection window 27 of the lid 20. Such optical coatings are well known in the art and can, for example, be formed by a multi-layer of dielectric films. The band pass filter substantially reflects light at the first wavelength and passes light at the second wavelength. Alternatively the detector 40 may be such that it is more responsive at the second wavelength than the first wavelength. For example the detector material could be transparent at the first wavelength and a high absorption region for the first wavelength, i.e. a beam dump, could be provided behind the detection region of each pixel in the array. In order to further improve the efficiency of the device in this embodiment the assay chip 30 itself may be provided with a dielectric coating that specifically reflects light at the second wavelength or band of wavelengths, but that is

transparent at the first wavelength or band of wavelenghts. If such an arrangement is used, fluorescent light emitted by the marker molecules away from the detector can also be captured by the detector chip 40.

If the light source is sensitive to back reflections, an optical isolator or circulator can be placed between the light source and the array chip.

Alternative to an arrangement where the bottom substrate 10 and the chip 30 are separate, the array chip 30 can be designed to fulfil both functions. In such a case the array chip 30 is made large enough to cover the substantially the whole perimeter of the lid. Again, fiduciary marks are employed to ensure that the chip will register with the lid 20 at a predetermined location, such that the array on the chip is aligned with the array of pixels in the detector. Whether it is more advantageous to provide the chip 30 and bottom substrate 10 as separate parts or one part depends on the design of the device and whether it is more important to save area of chip substrate in high volume applications, or simplify the design of the system.

In Fig. 3 and Fig. 4 further improved embodiments of the present invention are shown. In Fig 3 an array of lenses 70 is formed in a substrate 80, whereby the lens array is matched to the array of biological samples 32 and the array of pixels 41 in the detector chip 40 such that samples at a each vertex of the sample array are imaged on the corresponding vertex or pixel in the detector array by one corresponding lens in the lens array. Such an arrangement can provide a better spatial resolution as well as lower cross talk between adjacent vertexes in the sample array.

The lens array can be formed in a wafer-based substrate, e.g. silicon or silica. A mask of the array is produced on the substrate using known photolithography techniques and an isotropic etch is used to form spherical recesses in the substrate. The recesses are then filled by depositing a film of a second material. In case the substrate material is glass the second material can be a glass with higher refractive index than the glass of the substrate material. Techniques for varying the refractive index of glass are well known by means of doping.

Alternatively, if the substrate material is silicon, the second material may also be glass, but in this case a selective etch is performed from the side of the substate opposite that from which the first etch has been performed to form a recess at the location of each lens that takes away the silicon around the center of the lens and that stops at the curved glass structure of the second material deposited previously form the other side.

Subsequently, in either case described before the substrate is polished back to a planar surface on the side where the recesses were filled. If desired, the regions between lenses of the array can be doped separately to provide for additional optical isolation in the substrate between the lens regions. This will generally reduce the amount of light scattered between lenses and improve crosstalk in the system.

Using an array of lenses disposed between the array of samples and the array of detectors it is possible to further enhance cross talk isolation between vertexes of the sample array. In such an arrangement the crosstalk isolation can be further improved by providing spacer regions between the pixels of the detector. A trade off may have to be made between the most efficient use of the chip surface area and the level of cross talk to be achieved.

Instead of an array of lenses in a simplified embodiment an array of holes or vias may be formed in a substrate that is disposed between the array of sample and the array of the detectors. The substrate in which the holes or vias are formed is preferably opaque at the wavelength range to be detected. The crosstalk between adjacent vertexes in the array is thereby limited by the maximum angle at which light can pass through one of the holes to reach a given pixel on the detector. Although this arrangement does not provide for focusing this arrangement is a particularly simple solution for providing enhanced crosstalk isolation between adjacent vertices in the detector array.

When using the arrays of any of Figures 3, 4 or 5, the array may be located between the array chip 30 and the recess 21 of the flow cell or alternatively between the top surface of the flow cell and the detector chip 40.

In another embodiment the system of the present invention operates on the basis of in-line attenuation, rather than fluorescence. Here the binding reaction may be observed merely by the attenuation caused by a specific binding reaction on a vertex of the sample array, rather than the detection of fluorescently labeled ligands when light emitted from the light source onto the samples is attenuated when passing the samples on its way to the array of detectors. In such a situation it is preferable that the maximum of the responsiveness of the pixels in the detector is at the first wavelength or band of wavelengths.

In yet another embodiment of the present invention no primary light source is required and the biological or biochemical assay performed on the sample array is carried out on the basis of chemiluminescence or electrochemiluminescence of a tagged ligand. For example it would be possible to provide ligands with tagged luciferase or a similar chemiluminescent protein that will start to emit light when the flow cell is filled with a solution containing ATP.

Alternatively electrochemiluminescent ligands, e.g. ones that contain ruthenium metal ions, could be used, in which case it would be possible to design the assay chip such that it will be possible to apply an electrical field between the top of the flow cell and the chip. In such a case the assay chip is formed from a conducting substrate such as a doped semiconductor or a metal. An isolation layer may be provided between the assay chip and the lid isolating the assay chip from the lid electrically. The lid itself has conducting parts, such that a voltage can be applied between the conducting parts of the lid and the assay chip.

Operation of the flow cell:
In a first step the chip is engaged and clamped to the open lid of the flow cell, such that the flow cell is now closed. This step may be performed by an automated cassette-to-cassette chip handling system for high throughput applications. The flow cell is then washed with an appropriate wash buffer to remove any impurities and prime the flow cell. Subsequently the array is stained or hybridized with a buffer containing the staining or hybridization reagent. Once the staining or hybridization has taken place the flow cell is washed again either with the same or a different

wash buffer to remove excess staining or hybridization reagent. When this has taken place the measurement is carried out employing one of the methods outlined above, i.e. by using any of fluorescence, absorption, chemiluminescence, or electrochemi-luminescene. When the measurement has taken place either a new staining experiment can be performed on the same chip or a new chip can be used. If a new chip is used, the old one may be removed and replaced with a new chip by the automated handling system, preceded by a wash or purge step, clearing the flow cell of liquid. If the same chip is used again for another staining or hybridization, the flow cell may be washed first with a release buffer, releasing the staining reagent or hybridization reagent from its targets, followed by a further wash step. In some situations temperature variations may be used in conjunction with wash steps to remove the staining or hybridization reagents. Once the removal has been achieved a new staining and measuring step can begin, following the protocol outlined above.

In summary the present invention allows for a very compact design of a flow cell with low power consumption, due to high efficiency detection. It allows for fast screening of an assay array that does not require movement of an x-y stage. A z stage for focusing known in prior art devices employing confocal microscopy is also not required. Very small cell volumes can be achieved by using the micromachining methods described above which can accelerate fluid handling during operation of the flow cell. Furthermore the present invention allows screening of very large arrays, e.g. of millions of samples on a chip of one square inch. The invention further allows easy combination of the flow cell with automated chip handling systems, such as cassette-to-cassette handling. When wafer based micromachining techniques are used with the present invention, as described above, it is further possible to replace several seals normally requiring several sealing materials with mere pressure seals, thus reducing the complexity of the device further.

In another aspect of the present invention the flow cell system described can be used to count cell populations that bind to a specific marker, e.g. a phenotypic marker. In such a situation the bottom surface of the flow cell does not have to comprise a sample chip carrying a biological or biochemical sample. In this case

the bottom substrate may just be a transparent or opaque substrate, depending on whether a fluorescent, absorptive or luminescent method for detection is used.

If the detection method is by fluorescence the following protocol may be carried out: A suspension of cells of interest is stained using a staining reagent that is specific to the phenotypic marker of interest. For example, if one wishes to detect the percentage of CD8+ T cells in a sample of peripheral blood mononuclear cells (PBMCs) the cells may be labeled with a fluorescent tagged anti CD8 monochlonal antibody using methods well known in the art. Either the whole cell suspension sample or a fraction thereof is subsequently moved into the flow cell. The amount of cells in the flow cell at any one time is thereby controlled to be substantially less than the number of pixels or vertexes in the detector array, e.g. for a detector array with 3x10E6 pixels the number of cells in the cell may be 3x10E5. A measurement is taken by shining light of a first wavelength through the transparent bottom substrate onto the cell sample, which causes the fluorescent markers to fluoresce at a second wavelength. Labeled cells will appear as isolated dots on the array of pixels in the detector and the number of fluorescent dots can simply be counted out by electronic means. If the total number of cells in the sample has not been determined to start with, the detector array can be designed as a stacked array in which two detection regions are stacked vertically. The first of these two regions nearer to the sample may be responsive to the second wavelength of fluorescent light and transparent to the first wavelength of illuminating light. The second region is responsive only to the first wavelength of illuminating light. This may be achieved by engineering the optical band gaps of the materials from which the detection regions are formed using methods well known in the art.. With this detector design it is possible to detect labeled cells by means of fluorescence and unlabeled cells by means of in-line absorption at the same time. The ratio of labeled and unlabeled cells can hence be determined in a straightforward manner.

In order to immobilize the cells during the measurement process it may be desirable to label the cells with ligands that are attached to paramagnetic particles or beads. An external magnetic field, e.g. generated by a coil that is wound around the cell, can then be used to immobilize the cells on the bottom or top surface of the flow

cell. Once the measurement has been taken, the field can be switched off to allow the flow cell to be cleared of the cell sample by washing.

To optimize the system for such a cell counting application the height of flow cell may be selected such that, on average, cells in the flow cell will not overlap in their horizontal position by being stacked vertically.

It will be understood that in the above examples, the array of detectors is located sufficiently close to the array of test locations that no optical imaging system is needed to focus one image of the array of test locations, or even one image of a group of test locations, onto the array of light detecting devices. Each detector in the detector array is arranged to detect light from a single respective test location. The detection by the detector array is carried out simultaneously for a plurality of test locations until minimum cross talk between the signals from the separate test locations.

CLAIMS:

1. A method of analysing a biological or biochemical assay which method comprises outputting light simultaneously from a plurality of test locations in a test sample and detecting simultaneously optical signals from said test locations, said detecting comprising providing a plurality of optical detectors, one for each test location, and positioning said detectors in an array overlying the sample so that the position of each detector in the array has a one to one correspondence with its respective test location.

2. A method of analysing a biological or biochemical assay in a flow cell having a flow cavity enclosed by boundary walls one of which provides a flat output window transparent to optical signals to be output from the cell, which method is characterised by outputting light from a plurality of test locations in the assay to provide optical signals which are transmitted through the window of the flow cell and detecting said optical signals by incidence on respective light detectors in an array of light detectors forming an array of pixel locations respectively corresponding to said test locations and positioned close to said output window without requiring an optical imaging system to focus an image of the array of test locations onto the array of light detecting devices.

3. A method according to claim 2 in which said optical signals are generated from samples on a sample chip located in an aperture in a wall of the flow cell opposite said output window and parallel thereto.

4. A method according to claim 2 in which said light signals are generated from samples within the cavity of the flow cell, the depth of samples within the flow cell perpendicular to said output window being controlled to avoid more than one sample being stacked on the same output path.

5. Apparatus for analysing a biological or biochemical assay which apparatus comprises a support structure for a test sample having an array of test locations, said support structure comprising at least one planar surface, and said apparatus

further comprising an optical detector for detecting optical signals from said sample, the detector having a planar array of separate light detecting devices each corresponding to a pixel in the detector output and each having a position on the array corresponding to a respective desired test location, said planar array of light detecting devices facing said first planar surface substantially in parallel and in sufficient proximity thereto with said test locations being in between the said first planar surface and said array of light detecting devices, such that light from an array of test locations in the assay passes from the sample to a respective pixel location in the detector array without requiring an optical imaging system to focus an image of the array of test locations onto the array of light detecting devices.

6. Apparatus of claim 5 wherein said support structure comprises an output window transparent to said optical signals to be output from the sample and wherein said detecting devices are being located in a planar array in contact with said window

7. Apparatus of claim 5 wherein said support structure comprises an output window transparent to said optical signals to be output from the sample and wherein said detecting devices are being located in a planar array facing said window in close proximity thereto.

8. Apparatus of claims 5 to 7 wherein said detecting devices are located in a planar array with a pitch separation in the array corresponding to the pitch separation of test locations in the sample and the array of detecting devices being located such that the average distance between the planar array of detecting devices and the test locations is less than 5 times said pitch separation.

9. Apparatus of claim 8 in which the average distance between the planar array of detecting devices and the test locations is less than 2 times said pitch separation.

10. Apparatus of claims 6 to 9 in which said support structure includes a flow cell and said window is provided by a transparent wall region of the cell.

11. Apparatus of claim 10 in which a sample chip holding an array of biological or biochemical samples is releasably secured to the flow cell adjacent an aperture in a wall of the cell opposite said output window such that said samples are exposed in the cavity of the flow cell.

12. Apparatus of claim 10 or 11 in which the apparatus comprises a stack of planar elements in contact with each other at least some of said planar elements forming the flow cell and a further planar element providing the array of optical detectors.

13. Apparatus of claim 12 in which a sample chip forms one of said planar elements.

14. Apparatus for performing and reading out a biological or biochemical assay in a flow cell the flow cell being contained between an top surface, side walls and a bottom surface, the bottom surface comprising the top surface of a sample chip, the sample chip comprising biological or biochemical samples in an array characterized in that an array of optical detectors is directly apposed to the top surface of said flow cell to read out optical signals emitted from the samples on the sample chip such that substantially one vertex in the array of samples is imaged onto one vertex or pixel of the array of detectors.

15. Apparatus of claim 14 wherein the top surface of the flow cell is the bottom surface of a first substrate.

16. Apparatus of claims 14 or 15 wherein the bottom surface of the flow cell is the top surface of a second substrate.

17. Apparatus of claims 14 to 16 wherein the side walls are formed in a third substrate.

18. Apparatus of claims 14 to 17 wherein the flow cell comprises an inlet region, a central assay and detection region and an outlet region.

19. Apparatus of claims 14 to 18 wherein the features defining the side walls of the flow cell are micromachined into the third substrate.

20. Apparatus of claims 14 to 19 wherein the micromachined features defining the sidewall are formed by etching anisotropically through the third substrate.

21. Apparatus of claims 14 to 20 wherein the micromachined features defining the sidewalls are formed by anisotropically etching into but not through the third substrate.

22. Apparatus of claims 14 to 21 wherein a seal of the flow cell is formed by means of a pressure seal between the bottom substrate of the flow cell and the sample chip.

23. Apparatus of claims 14 to 22 wherein both the sample chip and the surface of the flow cell the chip connects with when engaged in the flow cell comprise fiduciary registration marks that can register with one another.

24. Apparatus of claims 14 to 23 wherein the detector chip directly abuts against the portion of the flow cell forming the sidewalls.

25. Apparatus of claims 14 to 24 wherein the sidewalls of the flow cell and the op of the flow cell are machined into a single substrate.

26. Apparatus of claims 14 to 25 wherein vias are machined through the substrate forming the top surface of the flowcell to provide for an inlet and outlet to and from the flow cell.

27. Apparatus of claims 14 to 26 wherein the vias are located towards opposite ends of the flow cell on either side of the central assay and detection region.

28. Apparatus of claims 5 to 27 wherein the array of detectors is a CCD device.

29. Apparatus of claims 14 to 28 wherein the portion forming the top surface of the flow cell and the portion forming the side walls of the flow cell are parts of the same monolithic component.

30. Apparatus of claims 14 to 29 wherein any or all of the components used to form the flow cell are made of silica.

31 Apparatus of claims 14 to 30 wherein any or all of the components used to form the flow cell are made of silicon on insulator.

32 Apparatus of claims 14 to 31 wherein any or all of the components used to form the flow cell are made of glass.

33 Apparatus of claims 14 to 32 wherein any or all of the components used to form the flow cell are made of quartz.

34. Apparatus of claims 5 to 33 wherein an array of lenses is disposed between the test locations and the detector, such that substantially one lens is provided for each vertex or pixel in the array of detectors.

35. Apparatus of claim 34 wherein the array of lenses is formed by etching a fourth substrate layer with an isotropic etch process and wherein the recesses formed in the isotropic etch process are filled with a material of higher refractive index than the refractive index of the material of the fourth substrate layer.

36. Apparatus of claim 35 wherein the filling with the second material of higher refractive index is performed through a chemical or vapor deposition process and the substrate is subsequently polished back such that the lenses substantially have one spherical refractive surface and one planar refractive surface.

37. Apparatus of claim 34 wherein the lens array is formed by etching a fourth substrate layer with an isotropic etch process and wherein the recesses formed in the isotropic etch process are filled with a material different from the material of the fourth substrate layer.

38. Apparatus of claim 37 wherein the fourth substrate layer is silicon.

39. Apparatus of claim 37 or 38 wherein the different material is silica.

40. Apparatus of claim 37 to 39 where a second selective etch is performed from the opposite site of the fourth substrate layer to form vias through the fourth substrate layer.

41. Apparatus of claims 34 to 40 wherein the lens array is formed in a silica substrate.

42. Apparatus of claims 34 to 41 wherein an optical coating is provided on one side of the lens array.

43. Apparatus of claims 14 to 42 wherein the flow cell has a height of 5 micron to 100 micron.

44. Apparatus of claims 14 to 43 wherein the flow cell has a height of 20 micron to 50 micron.

45. Apparatus of claims 5 to 44 wherein the pixels in the detector array are separated by non-detecting areas.

46. Apparatus of claim 45 wherein the non-detecting areas are substantially one half or less of the width of the detecting areas.

47. Apparatus of claim 45 wherein the non-detecting areas are substantially the same or less as the width as the detecting areas.

48. Apparatus of claims 14 to 47 wherein a light source projects light through the planar surface onto the samples on the sample chip.

49. Apparatus of claim 48 wherein the light source transmits light of a first wavelength or band of wavelengths and the detector detects light on a second wavelength or band of wavelengths.

50. Apparatus of claim 48 or 49 wherein the light of the first wavelength or band of wavelength causes fluorescence of the samples on the second wavelength or band of wavelengths.

51. Apparatus of claims 48 to 50 wherein the array of detectors is transparent to the first wavelength or band of wavelengths.

52. Apparatus of claims 5 to 51 wherein the array of detectors is provided with an optical coating on its front face.

53. Apparatus of claim 52 wherein the optical coating is reflective to the first wavelength of claim 50 and is transmissive to the second wavelength of claim 50.

54. Apparatus of claim 51 wherein an optical beam dump is provided behind the array of detectors, the beam dump absorbing the second wavelength.

55. Apparatus of claims 48 to 54 wherein an optical circulator is disposed between the light source and the assay chip

56. Apparatus of claims 5 to 47 wherein light is emitted from the array of samples by means of electrochemiluminescence.

57. Apparatus of claim 56 wherein the sample is chip is conducting.

58. Apparatus of claim 57 wherein an electrical field can be applied between the bottom and the top of the flow cell to cause the electrochemiluminescense.

59. Apparatus of claims 5 to 48 wherein light is emitted from the array of samples by means of chemical luminescence.

60. Apparatus of claim 59 where the chemical luminescence is caused by luciferase proteins.

61. Apparatus of claims 5 to 60 wherein a substrate having an array of vias or holes formed therein is disposed between the test locations and the detector, such that substantially one via or hole is provided for each vertex or pixel in the array of detectors.

62. Apparatus of claim 61 wherein the substrate in which the vias or holes are formed is opaque at the wavelength range to be detected.

23

The Patent Office

INVESTOR IN PEOPLE

Application No: GB 0027293.0
Claims searched: 1

Examiner: Diana Pisani
Date of search: 14 June 2001

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.S): G1A ACJF, ACJX, ADJM, ADJP, AKA, ARUL

Int Cl (Ed.7): G01N 21/59, 21/64, 21/76, 33/53

Other: Online: WPI, EPODOC, JAPIO

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	GB2351556 A	CAMBRIDGE IMAGING LTD., see especially page 10, paragraph 6 & page 13, paragraph 2.	1
X	GB2315131 A	CAMBRIDGE IMAGING LTD., see whole document.	1
X	GB2014300 A	OPTO ELECTRONIC DISPLAYS, see whole document.	1
X	WO99/58962 A1	IGEN INTERNATIONAL, see especially figures 3A, 3B, page 16, line 29 - page 17, line 14 & page 18, lines 20-28.	1
X	WO98/26277 A1	PROLUME LTD., see especially figure 11 & page 7.	1
X	US5779978	AVL MEDICAL INSTRUMENTS AG, see whole document.	1
X	US5736410	SRI INTERNATIONAL, see figures 31A, 31B & column 39, line 56 - column 40, line 12.	1
X	US5073029	EQM RESEARCH, INC., see whole document.	1

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

24

INVESTOR IN PEOPLE

Application No:	GB 0027293.0	**Examiner:**	Bob Clark
Claims searched:	2 and 14	**Date of search:**	11 October 2001

Patents Act 1977
Further Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.S): G1A (ACJF, ACJX, ADJM, ADJP, AKA, ARUL)

Int Cl (Ed.7): G01N 21/05, 21/59, 21/64, 21/76, 33/53

Other: Online: EPODOC, JAPIO, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	WO 99/58962 A1	(IGEN INTERNATIONAL) Line 12 p.15 to line 2 p.17, and line 11 p.52 to line 2 p.54	2,4
A	WO 98/13683 A1	(SARNOFF)	
X	US 5736410	(ZARLING et al.) Line 56 col.39 to line 24 col.40	2-4

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

DCID: <GB___2368903A__I_>

25

INVESTOR IN PEOPLE

Application No: GB 0027293.0
Claims searched: 5

Examiner: Bob Clark
Date of search: 11 October 2001

Patents Act 1977
Further Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.S): G1A (ACJF, ACJX, ADJM, ADJP, AKA, ARUL)

Int Cl (Ed.7): G01N 21/59, 21/64, 21/76, 33/53

Other: Online: EPODOC, JAPIO, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	WO 99/58962	(IGEN INTERNATIONAL) Line 12 p.15 to line 2 p.17, and line 11 p.52 to line 2 p.54	5-7,10,12
X	US 5736410	(ZARLING et al.) Line 56 col.39 to line 24 col.40	5-7,10, 12,13,56
X	US 5073029	(EBERLY et al.) Line 29 col.11 to line 15 col.12	5-9

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.